Supplementary Material (ESI) for Chemical Science



**Supplementary Information**

# Membrane protein biosensing with plasmonic nanopore arrays and pore-spanning lipid membranes

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## The detailed fabrication method of free-standing nanopore arrays.

### A. Low-stressed $Si_3N_4$ deposition and patterning

Starting from a (100) Si wafer, a 100 nm-thick low-stressed $Si_3N_4$ layer was deposited on the top and bottom sides using low pressure chemical vapor deposition (LPCVD). The topside nitride layer was then spin-coated (3000 rpm for 30 sec) with S1813 positive-tone photoresist (Shipley), followed by baking at 115 $^oC$ for 1 min. This protected the top surface from the following lithography process used to pattern the backside. The S1813 photoresist was spin coated on the Si backside surface at 3000 rpm for 30 sec and baked at 115 $^oC$ for 1 min. The desired patterns were obtained by exposing the photoresist through a chrome-on-glass photomask for 6 sec using a Karl Suss MABA6 contact aligner. After another baking at 115 $^oC$ for 1 min, the exposed resist was developed for 30 sec in a 20% solution (by volume) of 351 developer (Shipley). The $Si_3N_4$ layer on the backside of Si wafer was then etched with reactive ion etching (RIE, STS Model320) with 40 sccm of $CF_4$ and 4 sccm of $O_2$ gases at 100 W. The photoresist was then removed by sonication in acetone for 10 min.

### B. KOH anisotropic Si etching

Before KOH etching, the Si wafer was cleaned with a piranha solution, a 1:1 mixture of sulfuric acid and hydrogen peroxide, for 10 min and subsequently dipped in a buffered oxide etchant (BOE) for 10 sec to remove the thin chemical oxide. The wafer was then immersed in 25 wt% solution of KOH in water at 80 $^oC$. The Si wafer was etched until the $Si_3N_4$ layer was exposed from the backside of Si wafer, creating a free-standing $Si_3N_4$ layer with dimensions ranging from $50 \times 50$ $\mu m^2$ to $300 \times 300$ $\mu m^2$. The wafer was then cleaned in deionized water for 10 minutes to remove any residual KOH solution and dried with an $N_2$ stream.

**C. Metal deposition**

After KOH etching, a 5 nm Cr adhesion layer and a 200 nm-thick gold film were deposited onto the nitride layer with an e-beam evaporator (CHA, SEC 600). The deposition rates were 1 and 3 Å/sec for Cr and Au, respectively.

**D. Nanopore patterning and silica coating**

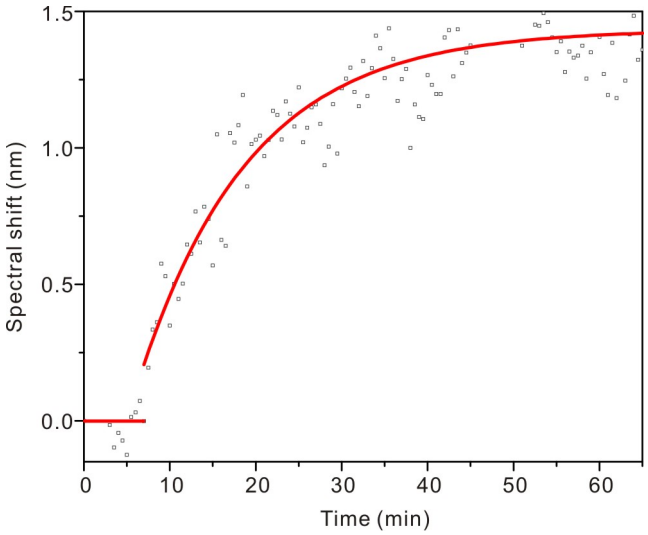
Nanopores through the Au and $Si_3N_4$ layers with a diameter of 200 nm were made into regular arrays using focused ion beam (FIB, FEI Quanta 200 3D) milling. The FIB conditions were 30 kV with a current of 500 pA. To facilitate versicle rupture, the gold surface was conformally coated with a 20 nm-thick silica layer using atomic layer deposition (ALD, Cambridge Nano Tech, Inc., Savannah) at 100 $^{o}$C. The deposition rate for each cycle as about 4 nm.

**E. Fabrication of PDMS microfluidic chip**

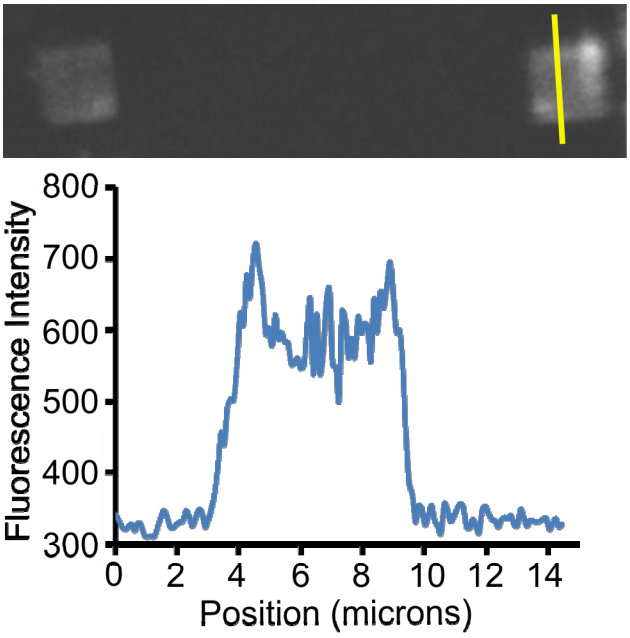
Soft lithography with polydimethylsiloxane (PDMS, Sylgard) was used to fabricate the microfluidic flow cell. The negative-tone master mold of the channel was patterned on a silicon wafer using SU-8 50 photoresist (Chembio), defining 50 μm deep and 1.3 mm wide channels. A 10:1 ratio of PDMS and curing agent was degassed in vaccum and cast to be 3 mm thick over the SU-8 photoresist pattern. After curing the PDMS at 60 °C overnight, the PDMS flow cell was cut from the master, and inlet and outlet holes were punched for tubing connections. After the PDMS was cleaned with acetone, IPA, and deionized water, the PDMS flow cell was aligned with the nanopore arrays using a contact aligner (Karl Suss MJB3). For the thin PDMS

membrane sealing the backside of the patterned Si wafer to create a bottom reservoir, uncured PDMS was spin coated on a plain Si wafer at 500 rpm for 10 sec. The following curing and cleaning processes were same as used for microfluidic flow cell.

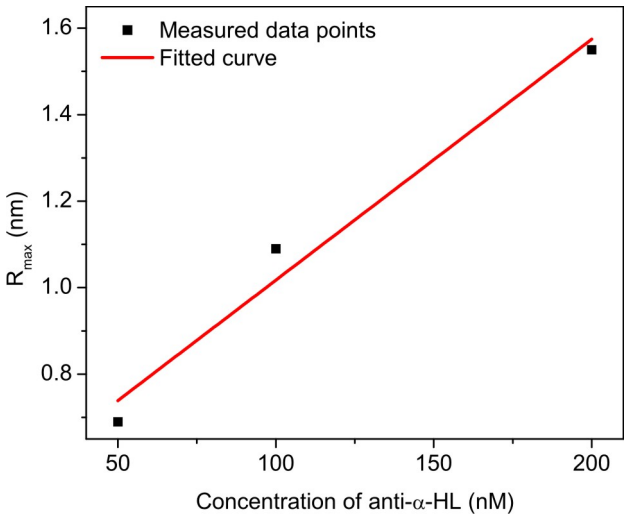
## Supplementary figures




**Supplementary Fig. S1.** A real time kinetic curve of the pore-spanning lipid membrane formation via vesicle rupture. A vesicle solution was injected after a 7 min baseline with a PBS solution and incubated for an hour.




**Supplementary Fig. S2.** Fluorescence image of the suspended lipid membrane from the underside of the nanopore array. The yellow line in the fluorescence image indicates the location of the line scan.




**Supplementary Fig. S3.** Maximum spectral shift response values obtained from Fig. 6b (black dots) and a linear fit of the data points to the concentrations of anti-α-HL used for the experiment.